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Effect of Parenteral Antibiotic Administration on Establishment of Intestinal Colonization in Mice by *Klebsiella pneumoniae* Strains Producing Extended-Spectrum β-Lactamases

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A mouse model was used to test the hypothesis that antibiotics with activity against anaerobes promote overgrowth of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strains in stool. Subcutaneous clindamycin consistently promoted establishment of high-density colonization, whereas piperacillin-tazobactam, ceftriaxone, and ceftazidime promoted colonization only when a large inoculum and/or more resistant strain was administered.

Use of broad-spectrum cephalosporins has been associated with the emergence of extended-spectrum β-lactamase (ESBL)-producing gram-negative bacilli (9–11). Although non-cephalosporin antibiotics have also been associated with ESBLs (7, 9, 14), the mechanisms by which these agents promote ESBL-producing organisms are not well defined. Because intestinal anaerobes provide colonization resistance against overgrowth of potential pathogens (2, 8, 13), we hypothesized that antibiotics that have been shown to reduce levels of intestinal anaerobes (i.e., clindamycin, piperacillin-tazobactam, ceftriaxone, and ceftazidime) (1, 2, 13) would promote overgrowth of ESBL-producing *Klebsiella pneumoniae* strains in mice, whereas antibiotics that minimally affect anaerobes (i.e., cefepime, levofloxacin, and aztreonam) (2, 5, 13) would not.

Two *K. pneumoniae* bloodstream isolates were studied. Strain P62 produces an SHV ESBL and P10045 produces TEM-1 and an SHV ESBL. The *bla* ESBL genes of both strains have mutations at amino acid positions 238 and 240, indicating that they encode SHV-5 or derivatives. The broth dilution MICs for P62 and P10045, respectively, were as follows: ceftazidime, 16 and 1,250 μg/ml; piperacillin-tazobactam, 4 and 156 μg/ml; ceftriaxone, 4 and 78 μg/ml; levofloxacin, <0.125 and 4 μg/ml; cefepime, 0.75 and 8 μg/ml; and aztreonam, 128 and 2,500 μg/ml.

The experimental protocol was approved by the Cleveland Veterans Affairs Medical Center's Animal Care Committee. Female CF1 mice (Harlan Sprague-Dawley, Indianapolis, Ind.) weighing 25 to 30 g were housed individually. On experiment day 0, esophageal inoculation of 10^3 CFU of ESBL-producing *K. pneumoniae* suspended in 0.5 ml of phosphate-buffered saline was performed with a stainless steel feeding tube (Perfektum; Popper & Sons, New Hyde Park, N.Y.). On experiment day 5, a second dose of 10^8 CFU was administered to all groups except the clindamycin group.

From experiment day −2 (2 days before the first ESBL-producing *K. pneumoniae* administration) through day 8, subcutaneous injection (0.2-ml total volume) of saline, clindamycin (1.4 or 16.8 mg/day), piperacillin-tazobactam (8 or 96 mg/day), ceftriaxone (2.0 or 12.4 mg/day), ceftazidime (3.0 or 36.8 mg/day), cefepime (2.0 or 24 mg/day), levofloxacin (0.375 or 4.7 mg/day), or aztreonam (3.0 or 37.5 mg/day) was administered at 12-h intervals. The lower dose of antibiotic was based on the daily dose recommended for human adults (in milligrams per kilogram of body weight), and the higher dose was the human equivalent dose calculated by the technique of Freireich et al. (6). The dose of levofloxacin was based on the 750-mg/day human dose. Stool samples were collected at baseline and at 2- to 7-day intervals after inoculation of ESBL-producing *K. pneumoniae*. The density of pathogens was measured as previously described, except samples were plated onto MacConkey agar (Difco Laboratories, Detroit, Mich.) supplemented with ceftazidime (10 μg/ml) (3).

The experiments were performed twice with a total of six or seven mice per group. For each experiment, randomly selected ceftazidime-resistant gram-negative bacilli from stool were subjected to speciation and susceptibility testing by VITEK (bioMerieux, Inc., Hazelwood, Mo.). For the lower-antibiotic-dose experiments, the density of colonization was monitored after discontinuation of antibiotic treatment to assess clearance of the ESBL-producing *K. pneumoniae* strains. Data were analyzed with SPSS version 10.0 (Chicago, Ill.). A Kruskal-Wallis test was performed to evaluate for significant differences among the groups on days 3, 5, 8, and 11. Because the data did not deviate substantially from normality, a one-way analysis of variance with a post hoc Scheffe correction to adjust for multiple comparisons was performed.

The effect of antibiotics on the establishment of colonization is shown in Fig. 1. At baseline, none of the mice had detectable ceftazidime-resistant gram-negative bacilli (level of detection, ~2 to 2.5 $\log_{10}$ CFU/g). After the 10^3-CFU inoculum of either strain (day 0), clindamycin at both dosages promoted persistent high-density colonization in comparison to the saline controls ($P < 0.0001$). The only other significant promotion of

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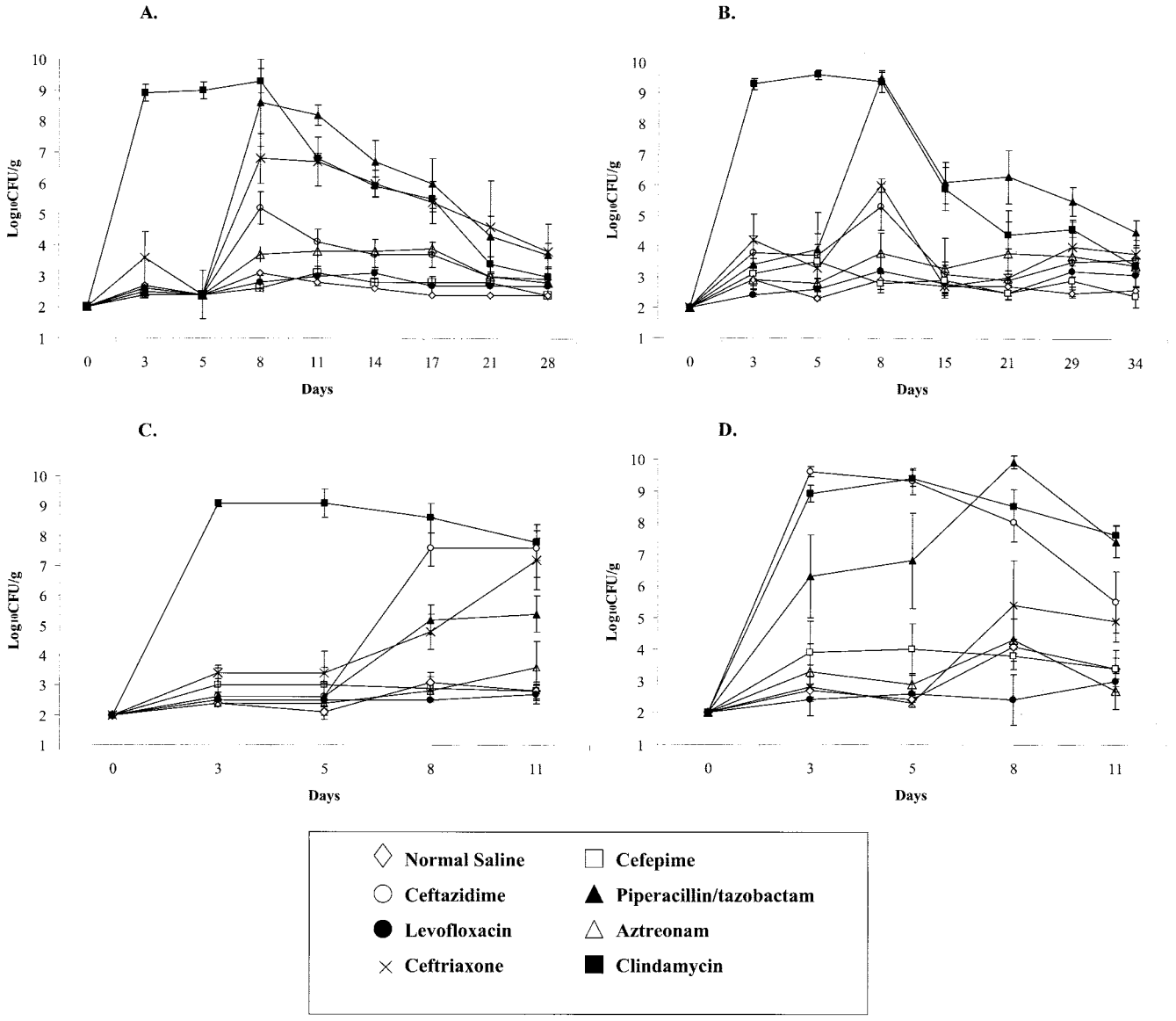

FIG. 1. Effect of subcutaneous antibiotic administration on the establishment of colonization with of ESBL-producing *K. pneumoniae* in mice. Densities of ESBL-producing *K. pneumoniae* in stool are shown for strain P62 with low-dose antibiotics (equal to human doses on a milligram-per kilogram basis) (A), strain P10045 with low-dose antibiotics (B), strain P62 with corrected human-equivalent doses of antibiotics (C), and strain P10045 with corrected doses of antibiotics (D). Mice received subcutaneous injections of antibiotics every 12 h from day −2 to day 8. Mice received 10^3 CFU of ESBL-producing *K. pneumoniae* by esophageal inoculation on day 0 and 10^8 CFU on day 5. If ESBL-producing *K. pneumoniae* organisms were not detected in stool, the lower limit of detection (~2 $\log_{10}$ CFU/g) was assigned.

overgrowth after the 10^3 inoculum occurred when the more resistant P10045 strain was administered in combination with higher dosages of ceftazidime ($P < 0.0001$ on days 3 and 5) or piperacillin-tazobactam ($P = 0.066$ on day 3 and 0.012 on day 5) (Fig. 1D).

After the 10^8-CFU ESBL-producing *K. pneumoniae* inoculum on day 5, the lower dosage of piperacillin-tazobactam promoted high-density colonization of both strains in comparison to the controls ($P < 0.02$ on days 8 and 11) (Fig. 1A and B), but at the higher antibiotic dosage, only the P10045 strain was promoted ($P < 0.001$) (Fig. 1D). After the 10^8-CFU inoculum, the lower dosage of ceftazidime promoted significant overgrowth of the P10045 strain in comparison to the controls ($P < 0.02$) and resulted in a trend toward increased density of the P62 strain ($P = 0.066$ on day 8); the higher dosage of ceftazidime promoted overgrowth of both strains ($P < 0.025$). After the 10^8-CFU inoculum, the lower dosage of ceftriaxone promoted overgrowth of both strains in comparison to the controls ($P < 0.02$), but at the higher dosage, only P62 was promoted ($P = 0.9$ on day 8 and 0.01 on day 11). After discontinuation of antibiotics, the density of colonization decreased for those treatment groups that promoted overgrowth (Fig. 1A and B). Aztreonam, levofloxacin, and cefepime did not promote colonization of either strain after the 10^3- or

10^8-CFU inoculums ($P > 0.05$ for all groups in comparison to controls). Analysis of several stool isolates of ceftazidime-resistant gram-negative bacilli yielded *K. pneumoniae* strains with susceptibility patterns identical to those of the original strains.

Our findings support the hypothesis that the indigenous anaerobic microflora inhibit colonization with *Enterobacteriaceae* such as ESBL-producing *K. pneumoniae*. Antibiotics with minimal activity against intestinal anaerobes (i.e., cefepime, aztreonam, and levofloxacin) did not promote ESBL-producing *K. pneumoniae* colonization, whereas an antianaerobic agent with negligible activity against *Enterobacteriaceae* (i.e., clindamycin) promoted high-density colonization. The effect of antibiotics such as piperacillin-tazobactam, ceftriaxone, and ceftazidime on establishment of colonization suggests a balance between inhibitory activity against ESBL-producing *K. pneumoniae* strains and promotion due to antianaerobic activity. For these antibiotics, overgrowth only occurred when a larger inoculum or more-resistant strain (P10045) was administered. Piperacillin-tazobactam demonstrated a similar inoculum effect with regard to vancomycin-resistant *Enterococcus* colonization in mice (4). It is notable that the higher dose of ceftazidime, but not ceftriaxone, promoted overgrowth of the P10045 strain; this may due to the higher level of resistance of P10045 to ceftazidime (ceftazidime and ceftriaxone MICs, 1,250 and 78 μg/ml, respectively) and greater biliary excretion of ceftriaxone (12).

Our findings have important clinical implications if validated in humans. A variety of antibiotics with antianaerobic activity are likely to facilitate transmission by promoting intestinal colonization with ESBL-producing *K. pneumoniae*. Antianaerobic antibiotics with minimal activity against ESBL-producing organisms (e.g., clindamycin) should be avoided when possible in outbreak settings. Formulary switches from broad-spectrum cephalosporins to antianaerobic agents with activity against ESBL-producing organisms (e.g., piperacillin-tazobactam) may be effective in part because the inhibitory activity of these agents may be sufficient to prevent the initial establishment of colonization. For patients with established high-density colonization, however, agents such as piperacillin-tazobactam may promote persistent high-density colonization and therefore transmission.

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